NEW INSIGHTS INTO THE PATHOGENESIS AND NON-SURGICAL MANAGEMENT OF GRAVES' ORBITOPATHY

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Abstract

Graves' orbitopathy, also known as thyroid eye disease or thyroid-associated orbitopathy, is visually disabling, cosmetically disfiguring and has a substantial negative impact on a patients' quality of life. There is increasing awareness of the need for early diagnosis and rapid specialist input from endocrinologists and ophthalmologists. Glucocorticoids are the mainstay of treatment; however, recurrence occurs frequently once these are withdrawn. Furthermore, in >60% of cases, normal orbital anatomy is not restored, and skilled rehabilitative surgery is required to reduce disfigurement, double vision and occasionally, to preserve vision. Clinical trials from over the past decade [Au: edits to define "recent" OK? Please edit my changes if I have misunderstood you This is fine] have shown that considerable benefit can be derived from the addition of anti-proliferative agents (such as mycophenolate or azathioprine) in preventing deterioration after steroid cessation. In addition, targeted biologic therapies have shown promise, including teprotumumab (anti-IGF-1R), which seems to substantially reduce proptosis, rituximab (anti-CD20), which reduces inflammation, and tocilizumab, which potentially benefits both of these parameters. Other strategies such as orbital radiotherapy have had their widespread role in combination therapy called into question. In the last decade, the pathophysiology of Graves' orbitopathy has also been revised with identification of new potential therapeutic targets. In this review we provide an up-to-date overview of the field, [Au: addition of linking text OK? This is fine] outline the optimal management of Graves' orbitopathy and summarise the research developments in this area to highlight future research questions and direct future clinical trials.

[H1] Introduction [Au: Please ignore H1, H2 etc. These are heading markers for our production department that will be removed in the final proofs. H1 headings can have a maximum of 38 characters, including spaces; H2 headings 38 characters; and H3 heading ~60 characters.]

Graves' orbitopathy [Au: We do not abbreviate terms that are fewer than 3 words, unless the term is within our style guide. GO is not in our style guide so I have removed it as an abbreviation. In addition, I find that manuscripts with fewer abbreviations are easier to read for non-experts Very happy with this change] is a rare complex autoimmune disorder which causes substantial morbidity[1]. The disorder can result in orbital disfigurement, double vision and even visual loss[2]. Consequently, Graves' orbitopathy has a substantial negative effect on quality of life[3], mental health[4] and socioeconomic status of patients[5,6]. The vast majority, more than 90% [Au: Can you please define "vast majority" by providing a figure (e.g. is vast majority 70% or 90% etc.)added] of patients with Graves' orbitopathy have Graves' disease, [Au: As with GO, I have removed GD as an abbreviation this is fine] an inflammatory autoimmune condition that is caused by thyrotropin receptor auto-antibodies (TSH-R-Ab)[7].

[Au: Large blocks for text can be difficult for readers to digest and so I have inserted a paragraph break here. If you disagree with the change, please feel free to revert. Agree with change]

[Au: I have made some edits to the following sentence for narrative flow. Please check that I have not altered your intended meaning. This is fine] Graves' disease is common throughout the world. The disease, which predominantly affects women, typically in their third to fifth decade[8], has an overall prevalence of 0.5%[8]. Around 15% of patients with Graves' disease who do not have Graves' orbitopathy at baseline will develop it, typically within 3-6 months on average. [Au: On average, how long does it take for patients to develop Graves' orbitopathy from baseline? (years or months etc.). I think our readers would benefit from this information. See my example linking text, but feel free to use your own text added] Approximately 2% of patients who develop Graves' orbitopathy will [Au: Addition for clarity OK? This is fine] develop moderate-severe disease[9,10]. A multicentre prospective study from 2018 [Au: We avoid the use of "recent" as it can be ambiguous for the reader. 2018 OK here? This is fine] proposed a predictive score of the risk of developing Graves' orbitopathy in Graves' disease with ocular inflammation at baseline; smoking, duration of thyroid dysfunction and especially the TSH-R-Ab titre were the four key risk factors[10].This score, however, was more useful at identifying [Au: OK? This is fine] individuals who would not develop Graves' orbitopathy during treatment for Graves' disease rather than predicting which patients were at risk of developing Grave's orbitopathy[10]. In October 2009, the Amsterdam Declaration, signed by over 80 organisations, proposed that the incidence and morbidity due to Graves' orbitopathy could be substantially reduced by preventive measures,

such as warning patients about the early symptoms of Graves' orbitopathy, stopping smoking, avoiding hypothyroidism after radioactive iodine administration [Au: Could you please provide 1 or 2 examples of preventative measures here? Text added] and improved early access to specialist care[11].

In order to best understand the treatments and pathogenesis of Graves' orbitopathy, it is important to know that most of the signs and symptoms of Graves' orbitopathy can be explained by the expansion of the orbital contents. The orbital fibroblast[2] is the target of a spectrum of autoimmune responses, which collectively promote proliferation, excess adipogenesis (formation of new fat cells by differentiation of fibroblasts) and over-production of extra-cellular matrix (ECM). The ECM comprises glycosaminoglycans (GAGs), such as chondroitin sulphate and hyaluronan, which is not sulphated but is able to absorb up to 1000 times its weight in water[12].

Glucocorticoids are the mainstay of treatment in Graves' orbitopathy, however, the mechanism of action of high doses remains largely unexplained, and this needs to be balanced against their adverse effects[13] . Although glucocorticoids are known to reduce inflammation and deplete leucocytes, a direct action on the adipocytes including the inhibition of 'browning' processes in orbital fat cells is possible[14]. In addition, studies indicate 11beta hydroxysteroid-Dehydrogenase [Au: OK? This is fine] expression might [Au: "May" can be ambiguous to the reader, we prefer to use "Might", "Could," "Can" etc. This is fine] also have a role in the development of Graves' orbitopathy, possibly by local induction of adipogenesis [15,16]. In the last decade, our understanding of the pathogenesis and treatment of Graves' orbitopathy has improved as a result of both clinical trials and laboratory research.

In this review we provide a detailed overview of the clinical and surgical management of Graves' orbitopathy. We have focussed on trials from the past 3 years whose results provide insight into the pathogenetic mechanisms in operation. Finally, we deal with disease pathogenesis more broadly, to highlight possible future clinical trials and improvements to patient management.
[Au: Can you please include a paragraph that sums up the aims of your Review. I have provided a few example 'sentence starters' that you can use, but please feel free to also suggest your own Sentences added.]

[H1] Assessment of Graves' orbitopathy

Due to the complex pathogenesis of Graves' orbitopathy both visual function and cosmetic visual appearance [Au: by 'visual appearance' do you mean the physical appearance of a patient is affected or that the way in which a patients' vision appears to them is affected? Can you please specify made clearer] can be affected; therefore several validated assessment scores are used to assess different components of the condition (**Box 1** [Au: Of note, I have moved all of the boxes to the end of the manuscript so that they remain with the other display items This is fine]). The two main current Graves' orbitopathy classifications are from the European group on Graves' orbitopathy[17] (EUGOGO) and Vision, Inflammation, Strabismus, Appearance (VISA)[18]. [Au: Edits to the following sentence to improve clarity. Please check that I have not altered your intended meaning and feel free to edit my changes if I have misunderstood you changes are great] EUGOGO is the most common classification used in Europe, whereas VISA is the most common in USA and [Au: We avoid "/" in this context. is "and/or" OK here?slight change] Canada. Other scores include Clinical Activity Score (CAS)[19] which focusses on the degree of inflammation, NOSPECS[20,21] which is a useful mnemonic for assessing severity[20,21] and Ophthalmopathy Index which assesses inflammation and visual function[22]. [Au: Could you please introduce the other activity and severity scores in the opening paragraph of this section? e.g. Clinical Activity Score, NOSPECS and Ophthalmopathy Index. I think it might also be useful to introduce VISA-V,S,A (and define V,S,A) scales and VISA inflammation index as it is currently unclear if these are both part of VISA or separate entities. Added other scores here VISA added below]

[Au: Paragraph break added here]

The EUGOGO classification of disease severity is sight-threatening, moderate to severe and mild Graves' orbitopathy. The aims of EUGOGO were to investigate newly introduced drugs on severity, signs and symptoms as well as on clinical activity. In addition, the validated EUGOGO quality of life questionnaire is a further relevant pillar to evaluate the efficacy of the tested drug. EUGOGO has introduced one score each for clinical activity (CAS) and severity (CSS)[23]. The CAS component encompasses subjective symptoms i.e. pain and inflammatory signs i.e. swelling and redness. In comparison, CSS evaluates the magnitude of the exophthalmometer or proptosis values, lid retraction, diplopia grades and corneal involvement. The more "simplistic" VISA classification uses four signs only (Visual acuity, Inflammation, Strabismus or motility disturbances and Appearance) and therefore does not directly correlate with the EUGOGO CAS and CSS. A one-to-one direct comparison of both classifications is pending. It is therefore worth

noting that EUGOGO and VISA are not interchangeable[24].[Au: Could you please add a similar description of the VISA classifications? I.e. what is the classification of disease activity in VISA? Are the VISA classifications split into VISA inflammation index and VISA-V,S,A score? If so, I think this should be explained here. More detailed explanation provided] [Au: why is this? Could you briefly explain. Is it because one classification system see above]. This distinction [Au: "This" needs to be followed by a noun. Distinction OK here? If not, please feel free to choose your own This is fine] is particularly important when interpreting their assessment of treatment responses in clinical trials.

The Clinical Activity Score[19] and the VISA Inflammation index component of the VISA classification [Au: OK? This is fine] [18] reflect disease activity, whereas the EUGOGO[17,25], NOSPECS [20,21], VISA-V,S,A scales[18], and Ophthalmopathy Index[22] can all be used to assess severity including ocular deformity and visual dysfunction. The relative merits of these scores were [Au: Can you please define "recently" here by providing a time frame?] assessed elsewhere in 2015[24].

EUGOGO patient reported outcomes are also used to assess the effect of Graves' orbitopathy [Au: addition of Graves' orbitopathy for specificity OK? This is fine] on patient quality of life – the internationally acknowledged disease specific Graves' orbitopathy Quality of Life questionnaire (GO-QOL) is a validated outcome for both visual function and cosmetic visual appearance [Au: Can you please define "appearance" here. Is this the appearance of the patient or the appearance of their vision changed?] [26,27]. There is now some evidence that appearance has a greater negative effect on anxiety and depression than visual function[28]. [Au: Can you please define "recent" by providing a timeframe?] Trials in 2018[29,30] used other composite primary outcome scores based on several factors including diplopia score, range of eye movements and degree of proptosis.

In contrast to Clinical Activity Score, which was validated in numerous trials, the VISA score, although easy to document, has not been applied in prospective randomized trials up-to-now. A one-to-one comparison of these two assessments is worthwhile and warranted.

[H1] Early identification of Graves' orbitopathy

Increases in the awareness of Graves' orbitopathy within the clinical community and earlier diagnosis have resulted in improvements with regards to promptness and appropriateness of referrals to specialist centres[31]. [Au: Edits here for clarity, please check that I have not altered your intended meaning. In addition, could you please be specific regarding the improvements. Does this mean more patients are being referred or that the patients are being triaged more quickly and efficaciously?Made slight change above] In response to the Amsterdam Declaration, the UK TEAMeD *5* approach (http://www.btf-thyroid.org/TEAMeD-5)[32] [Au: Can you please add this website to the reference list? I don't want to disrupt the formatting by doing it myself. The format for citing websites should be: Author. Title of online article. Website name. http address (2015).Added] has been developed for use by endocrinologists. This approach can be used to detect Graves' orbitopathy early [Au: Changed "earlier" to "early" as "earlier" required a comparator this is fine] and improve outcomes for patients with Graves' orbitopathy. TEAMeD's five steps are shown in **Box 2**. Since >80% of cases of Graves' orbitopathy arise at the same time or after the diagnosis of thyrotoxicosis[33], targeting endocrinologists should enable early detection of the condition and/or institution of preventive measures in the majority of cases of Graves' orbitopathy.

[H1] Medical management of Graves' orbitopathy [Au: I have added some more subheadings to this section as I feel this helps improve the flow. Please feel to remove them if you disagree. We agree the flow is improved]

Simple medical management strategies, such as stopping smoking, use of selenium supplements [Au: Can you please provide a few examples here please?added] can be effective in limiting the disease. Restoration and maintenance of euthyroidism is essential [in preventing disease occurrence and progression Au: essential for what, specifically? Disease treatment or prevention etc.?] as both hyperthyroidism and hypothyroidism have a negative impact on Graves' orbitopathy[23]. [Au: I've added the following sentence to alter the narrative flow – introduce the simple strategies and then the more complex ones. Please feel free to alter or edit my addition if I have misunderstood your intended meaning slightly altered] Of the three management strategies for hyperthyroidism (anti-thyroid drugs, surgery and radioactive iodine) anti-thyroid drugs especially and surgery do not influence the natural course of Graves' orbitopathy (beyond restoration of euthyroidism), whereas radioactive iodine treatment confers an increase in risk of developing or exacerbating Graves' orbitopathy; although this risk [Au: "risk" to define "this" OK? This is fine] can be mitigated by concomitant steroid therapy [34]. Although not-evidence-

based, many clinicians who use anti-thyroid drugs opt for 'block and replace' therapy to optimise thyroid status in Graves' orbitopathy.

[H2] Smoking status [Au: I feel that this section could be improved with some guidance for clinicians. E.g. Do you feel clinicians should advise their patients to stop smoking and provide assistance for patients who do smoke? Change below]

Clinicians should strongly encourage patients with Graves' disease and those with Graves' Orbitopathy to stop smoking. Smoking is a major risk factor for Graves' orbitopathy since it contributes to both its development and progression[35,36], and is associated with increased severity[37]. Furthermore, smoking negatively effects the efficacy of immunosuppressive treatments for Graves' orbitopathy[38,39] and smoking cessation can improve Graves' orbitopathy outcome[40]. Smoking components might induce adipogenesis and synthesis of GAG, as suggested in an *in vitro* model of Graves' orbitopathy; however further evidence is needed to test this hypothesis *in vivo*[41]. Smoking in patients with untreated Graves' orbitopathy seems to be associated with an increase in extraocular muscle volume, but not orbital fat volume[42]. Whether vaping will have some degree of negative impact on Graves' orbitopathy remains to be elucidated as nicotine induces release of pro-inflammatory cytokines[41].

[H2] Ocular lubricants

Ocular lubricants and deliberate eye closure during prolonged visual tasks (such as, reading or use of visual display units) will alleviate symptoms and protect from corneal damage. Selenium supplementation (100 mcg twice a day) has been shown to be effective in stabilising mild Graves' Orbitopathy during treatment as well as during a six-month follow-up[43]; [Au: effective for what, specifically? Added] however we are still waiting for data on its use in Graves' orbitopathy prevention. Although widely used, there is lack of evidence of benefit for using selenium supplementation in patients with moderate to severe Graves' orbitopathy or inactive disease.

[H2] Glucocorticoids

Glucocorticoids are the mainstay of treatment for active disease, but there is a clear need to identify new therapeutic strategies. Recent randomised clinical trials indicate that intravenous methylprednisolone given weekly at starting doses of 0.5 g per week for six weeks then 0.25 g per week for another six weeks (cumulative dose of 4.5 g) seems to optimise the balance between efficacy and side-effects and is associated with fewer adverse events [Au: I have changed "better tolerated" for "adverse events" to remove the onus from patients (the idea that it is them who are

not tolerating a treatment) on to the treatment as it might not work for them. Edits OK? This is fine] than oral high dose glucocorticoids[23]. Doses higher than 4.5 g and more frequent doses than once a week [Au: edits here to add comparators OK? This is fine] can be used in severe or sight-threatening disease; however, reducing relapse rates and optimising final outcomes seems to require combinations of current therapies, given that active disease might last 1–2 years, and recurrence at the time of glucocorticoids withdrawal often occurs[44-47] (key therapeutic agents are summarized in **Table 1**). In 2018 the CIRTED (Combined Immunosuppression and Radiotherapy in Thyroid Eye Disease N=126) and the EUGOGO led MINGO (Mycophenolate in Graves' orbitopathy for MINGO or Mycophenolate plus methylprednisolone versus methylprednisolone alone in active, moderate-to-severe Graves' Orbitopathy N=164) [Au: Can you please define recently? changed] identified anti-proliferative treatments (azathioprine and mycophenolate), which have a role in improving outcomes, although mycophenolate is associated with markedly fewer adverse events [Au: OK? This is fine] [29,30].

In particular, the large [Au: Can you please state the n of these trails either here or above? Changed above] EUGOGO led MINGO trial[30] implies the potential advantage of combining IV steroids to a non-steroidal anti-proliferative drug pertaining to better and sustained response. The findings were confirmed by a large (N=174) [Au: Could you please define the n for this trial? added] randomised trial from China which also reported the beneficial effect of mycophenolate[48]. However, in both studies[30,48] [Au: of which study, specifically? Both studies – text changed] the drug had no clinically relevant effects on proptosis [G] [Au: I have highlighted suggestions for glossary terms throughout your manuscript with a [G]. Please provide succinct, one-sentence definitions for these specialist terms in the space provided at the end of the document. Added] and/or diplopia [G done]. Further, rehabilitative surgery was often required after the immune-suppressive therapy was stopped.

With regards to the CIRTED trial[29], although post-hoc analysis showed a potential beneficial effect of combining steroids and azathioprine drawing definitive conclusions is limited by the high number [Au: what proportion of participants dropped out? Text added] of dropouts. Although 103 of the 126 trial participants (81.2%) provided outcome data, 84 (66.6%) completed their allocated treatment of radiotherapy or sham radiotherapy and only 57 (45.2%) continued to take azathioprine or placebo up to 48 weeks (therefore dropouts had less [Au: less than what? This statement requires a comparator] effect on radiotherapy than azathioprine as most had received radiotherapy prior to withdrawal). Studies from the 1980's [Au: Could you please define

older by providing a timeframe?] suggested similar benefits to azathioprine might be observed with cyclosporin[49], and cyclosporin has subsequently been widely used for moderate to severe disease. Current guidance for the initial management of Graves' orbitopathy is summarized in **Figure 1**[23,50].

The aim of treatment of Graves' orbitopathy (**Box 3**) is to suppress orbital inflammation and reduce consequent tissue re-modelling in extraocular muscles, orbital fat and other periocular soft tissues[51,52]. Traditionally, glucocorticoids have been the treatment of choice for active disease. Methylprednisolone inhibits key pathological factors including prostaglandin secretion, fibroblast activity, GAG production[53], as well as the expression of pro-inflammatory proteins in the orbital tissue as shown in a proteomics study[54]. Furthermore, high dose methylprednisolone reduces the number of circulating dendritic cells and decreases TSHR-Ab levels[45], which have a key role in driving the activity and severity of Graves' orbitopathy.

The use of local, low dose (10 Gy) [Au: Could you please define "low dose" by providing a figure? done], orbital radiotherapy in Graves' orbitopathy yields less irradiation-induced short and long-term side-effects[55] although remains controversial. In randomized trials that include patients with mild or moderate-to-severe Graves' orbitopathy [56,57], radiotherapy as a single agent was equivalent to oral prednisolone[57] and significantly (p=0.02) [Au: Can you please provide a *P* value for the use of "significantly" here? If not, we prefer the use of "markedly", "notably" etc. changed] better than sham irradiation[56]. Furthermore a randomized trial of low dose (10 Gy) versus high dose (20 Gy) in moderate to severe Graves' Orbitopathy showed low dose radiotherapy was at least as effective and much better tolerated than high dose treatment[55,58]. In this three arm study protracted low dose therapy for 20 weeks was the preferred treatment by patients[57].

However a [Au: Can you please define the use of "recent" here by proving a timeframe? changed] trial in 2001 showed less clear benefit from radiotherapy and produced conflicting results, possibly due to treatment of disease that was beyond the active phase or the non-randomised or masked study design[59]. The CIRTED study made particular effort to focus on participants with active disease, including a two week trial of steroid response, and showed no additional benefit when orbital radiotherapy was added to high dose oral glucocorticoids[29].

To date, no randomized trials of radiotherapy plus intravenous glucocorticoids as the standard regimen have been reported[29,59]. However, as shown in the Mourits double-blind trial[60] and in several other randomized trials[56,57] and recommended in the ETA guidelines[23] radiotherapy is likely to have a role in patients with diplopia and motility disturbances . In a 2018 [Au: Can you please define "recent" here? changed] review[61], it was proposed that orbital radiotherapy might help potentiate the mechanism of high doses of steroids leading to inactivation of the disease. However, the lack of additional efficacy with high dose oral steroids in the CIRTED trial[29] suggests that radiotherapy should not routinely be used in combination with high dose steroids. It should be noted that CIRTED did not address the benefit of radiotherapy alone in patients with active/severe eye disease and disturbances of muscle motility and/or diplopia as recommended in previous guidelines[25]. The CIRTED study also did not directly answer the question of whether radiotherapy confers additional benefit to patients receiving pulsed intravenous steroid, although this would seem unlikely given the lack of additional benefit with the lesser amount of steroid administered during oral dosing in CIRTED.

[H2] Surgical management of Graves' orbitopathy

Despite medical advances, surgery has an important role in the treatment of Graves' orbitopathy. Surgery is required in the acute phase of the disease where there is immediate risk to vision (optic neuropathy). The optic nerve is vulnerable to compression and vascular compromise as it enters the orbit, where it is crowded by the origins of the surrounding extraocular muscles. Compression of the optic nerve [Au: This needs to be followed by a noun, could you please define "this" here? changed] occurs in 3–5% of patients[62] and cases where optic neuropathy is not responding to alternate high doses (750 mg/day 3 days per week) of intravenous steroids for two consecutive weeks will require orbital decompression[23]. The majority of surgery is rehabilitative (**Box 4**).

[H1] Mechanistic insights from recent clinical trials.

The key clinical trials are summarized in **Table 1,** which highlights the variable effect of altering different pathways in the pathogenesis of Graves' orbitopathy. These pathways are summarized in **Figure 2**. A summary of the doses and monitoring requirements for agents utilised in Graves' orbitopathy since 2015 [Au: "Newer" required a comparator. Could you please provide a date here instead? changed] is shown in **Table 2**.

[H2] Mycophenolate

Mycophenolate mofetil (MMF) is used in combination with glucocorticoids to prevent allograft rejection[63]. MMF substantially inhibits proliferative responses of T-lymphocytes and B-lymphocytes to both mitogenic and allospecific stimulation and antibody formation by B-lymphocytes[64]. Furthermore, it prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion to endothelial cells and inhibits recruitment of leukocytes into sites of inflammation[65,66]. [Au: Please reference this statement.done] MMF also potentially modulates the chemotaxis of infiltrating activated lymphocytes in inflammatory tissue[66]; however, [Au: could you please define "recent" here by providing a timeframe? changed] data from 2019 also indicate that MMF can act at the level of the phosphoinositide 3-kinase (PI3K) and mammalian target of rapamycin (mTOR) pathways in non-lymphoid cells, offering a mechanism of direct effect on orbital fibroblasts[67] (**Figure 2**).

In 2017, and 2018 [Au: Could you please define the use of "recent" here by providing a timeframe? changed] two studies [Au: Could you please provide the n for each of these studies?already done earlier it's the MINGO and YE study] prospectively and randomly tested mycophenolate versus steroid[30,48]. In the 2017 Chinese study[48] (in which the authors tested [Au: OK? This is fine] MMF) improvements were reported in Clinical Activity Score, [Au: OK? This is fine] diplopia and proptosis, although this was not observer masked [G: [Au: Could you please define "observer masked" here? Thought best to add to glossary].

The observer-masked European study[30] [Au: Could you please provide the n number here? Mingo study again] (in which the authors investigated [Au: OK? This is fine] Mycophenolate sodium) revealed additional benefits of mycophenolate. This trial[30] showed in a post-hoc analysis a better outcome in terms of overall ocular improvement at week 24 (end of intervention) and week 36 (three-month follow-up) in the group receiving combination therapy compared with the group that were treated by intravenous glucocorticoids alone. However, the authors also reported that relapses occurred in both groups at the same extent. Additional benefits were noted in the combined treatment group (steroids + mycophenolate sodium), in detail better results were observed in the combined treatment group pertaining to overall ophthalmic improvement at weeks 24 (p=0.03) and 36 (p=0.02), CAS at week 12 (p=0.04), downgaze duction (eye muscle motility when looking downgaze) at week 36 (p=0.009), eyelid swelling (week 36, p=0.027) and caruncle swelling (week 36, p=0.030). [Au: Could you please detail these benefits? changed] [Au: Could you please explain what downgaze duction and elevation are? Changed also] ,

[H2] Azathioprine

Azathioprine is an anti-proliferative agent with similar mode of action to mycophenolate. Although ineffective as monotherapy to treat Graves' orbitopathy [68], it could be a potential adjunct to steroid treatment[29], and like MMF, potentially could act directly on signalling pathways in the pre-adipocyte. Data from the CIRTED trial[29] identified potential benefits with regard to reduced relapse in features of Graves' Orbitopathy [Au: were these Graves' orbitopathy relapse rates? Can you please specify changed] after withdrawal of steroid although it is less well tolerated than MMF[29]. It is important to note that in the CIRTED trial 66% of participants were allocated to azathioprine and 45% of those allocated to placebo did not complete 48 weeks of treatment[30]. However, the majority of patients did return for review (82%), which strengthens the validity of the study, with no baseline bias in the returners between groups.

Despite low adherence rates, even in intention to treat, the point estimate for odds ratio for improvement for patients treated with azathioprine [Au: Addition for clarity OK? This is fine] was substantial - 2.56 (95% CI 0.98–6.66, p=0.054). In a sensitivity analysis in which patients who withdrew during the trial were recoded to unfavourable outcomes regardless of their status at 48 weeks, the effect of azathioprine treatment was enhanced (OR 3.65, 95% CI 1·34–9·86, p=0.011). Furthermore, in a post-hoc analysis of patients who completed their allocated therapy the OR for improvement was very large; 6.83 (1.66–28.1, p=0.008). No significant improvement was observed in GO-QoL compared to placebo — the major benefit appeared to be in reducing the relapse rate after steroid withdrawal. These findings are therefore consistent with a role for azathioprine as a steroid sparing agent. As the CIRTED trial[29] used oral rather than intravenous glucocorticoids, conclusions can also not be drawn on the role of azathioprine and intravenous glucocorticoids. Taken together, the data suggest MMF should be the preferred anti-proliferative agent in Graves' orbitopathy based on tolerability and probable efficacy.

[H2] Teprotumumab

Teprotumumab is a recombinant, fully human monoclonal antibody of the immunoglobulin G1 (IgG1) subclass. This monoclonal antibody binds to the insulin-like growth factor 1 receptor (IGF-1R) with high affinity, behaving as a pharmacological, functional inhibitor, blocking the activation of IGF-1R by its endogenous ligands (IGF-1 and insulin-like growth factor 2, IGF-2) and causing internalization of the receptor[69,70]. Teprotumumab, results in a complete shutdown of IGF-1R signaling and importantly shows no functional agonistic activity.

This blocking of the IGF-1R was predicted to have substantial benefits in Graves' orbitopathy as active (inflammatory). Graves' orbitopathy has been reported to be triggered and driven by autoimmune activation of orbital fibroblasts by autoantibodies with receptor agonist properties that signal through IGF-1R-dependent mechanisms[71] (**Figure 2**; discussed in more detail later in the review). In particular, the activation of orbital fibroblasts by autoantibodies stimulates the release of chemoattractant cytokines, promoting T-cell infiltration into orbital tissues[72]. This infiltration [Au: addition of "infiltration" OK? Please feel free to edit my changes if I have misunderstood you this is fine] triggers a local inflammatory response which, combined with the autoimmune driver, results in proliferation and differentiation of fibroblasts, tissue expansion, increases in extracellular matrix, edema and extensive remodeling of orbital tissues (**Figure 2**)[70,71,73]. Teprotumumab will therefore fully block these pathophysiological responses. In keeping with this, the main impact of teprotumumab on Graves' orbitopathy seems to be in markedly reducing proptosis and clinical activity score, including improvements in double vision[73]. The initial impressive results with teprotumumab, which is not yet approved by regulatory authorities, may well be a 'game-changer' but need to be replicated in future studies and compared with intravenous glucocorticoids.

[H2] Rituximab

Rituximab is a monoclonal antibody that targets CD20, which is only expressed by B cells, and is present from the stage of pre-B cells to mature and memory B cells, but not on the plasma cells that ultimately produce antibodies[74]. [Au: Please reference this statement. done] In 2015 [Au: Can you please define recent here by providing a timeframe? Have modified the sentence] 2 clinical trials[75,76] reported contradictory outcomes; no benefit in 1 trial when compared with placebo[75] however significant (P=0.006) [Au: Can you please provide a P value to go with your use of "significant" here? added] improvement in clinical activity score compared with IV steroids[76]. A reanalysis of the data implied that rituximab is most effective when administered early to patients with active disease[77]. Although rituximab depletes B cells, it does not necessarily reduce TSHR-Ab levels, suggesting other modes of action, for example such as a reduction in antigen presentation and activation of T cells[78]. More data and larger randomized trials are warranted prior to a definitive recommendation of the drug in patients with active and severe Graves' Orbitopathy.

[H2] Tocilizumab

A preliminary study reported that tocilizumab, a humanized monoclonal antibody against the interleukin-6 receptor, showed efficacy in terms of activity reduction when used off-label in Graves' orbitopathy refractory to intravenous [Au: OK? This is fine] glucocorticoids[79]. In addition, a subsequent case report that included two patients confirmed efficacy [Au: Was this efficacy confirmed in the same terms? That is in terms of activity reduction when used off label? yes][80].

More recently in 2018, [Au: Could you please provide a timeframe here? done] a multi-centre randomised double masked trial in Spain identified that the use of tocilizumab in glucocorticoid resistant Graves' orbitopathy resulted in an almost [Au: For the next points, "higher" odd, "greater chance" and "greater improvement" could you please specify what tocilizumab was compared with in the trial?added] 10-fold higher odds of having a reduction in clinical activity score of at least 2 points, a greater chance of having a clinical activity score less than 3, greater improvement in EUGOGO ophthalmic score and a reduction of exophthalmos than placebo[81]. These impressive initial findings need confirmation in a larger clinical trial as concerns have been raised from this study due to the small numbers of patients included (N=32), [Au: How many patients were included in the study?added] who were also heterogeneous with regard to their Graves' Orbitopathy [Au: TED used here, is my definition OK? Fine but Graves' Orbitopathy also fine] severity, pre-treatment with a variety of immunosuppressive drugs and not all the required ophthalmic data were clearly stated[81].

[H2] Other novel targeted therapies

Belimumab has been used off-label for Graves' orbitopathy, but well-designed focused randomized controlled trials are needed before drawing any definitive conclusion about its efficacy at treating the disease [Au:OK? This is fine][51]. Belimumab is a monoclonal antibody directed towards the B cell activating factor, and similar to rituximab, belimumab targets B cells, especially naïve and transitional B cells. It is currently licensed for systemic lupus erythematosus [Au: Definition of SLE OK? This is fine] [82] and clinical trials in Graves' orbitopathy are currently ongoing[51].

Alternative non-immune therapies have been considered for the treatment of Graves' orbitopathy. Bimatoprost, a treatment for glaucoma, has been reported to cause enophthalmos

and laboratory evidence suggests it inhibits proliferation and differentiation of orbital fat cell precursors[83]. Although a 2019 [Au: Could you please define "recent" here by providing a time frame?altered] clinical trial in stable, late, inactive Graves' orbitopathy revealed no evidence of benefit[84], trials in early, active disease are warranted.

[H1] Pathogenesis of Graves' orbitopathy [Au: as level 1 (H1) headings can only have a maximum of 38 characters, including spaces, I have edited this heading. Changes OK?]

[H2] New insights on pathogenesis [Au: I have added this new subheading here as I feel that the sections below fit well into a section on pathogenesis. What do you think? Please feel free to edit my changes if you disagree.]

The focus of studies into the pathogenesis of Graves' Orbitopathy[Au: Which studies specifically? Into the pathogenesis of Graves' orbitopathy?changed] has been largely on the orbital fibroblast as these are key in the early pathogenesis of Graves' orbitopathy.[Au: Could you please define recent here by providing a time frame? added] Studies reported in 2015 have indicated the mesenchymal-stem cell (MSC) properties of orbital fibroblast, using both *in vitro* lineage specific differentiation protocols and phenotyping by flow cytometry. Notably orbital fibroblasts are able to undergo adipogenesis, which probably predominates in the manifestation of Graves' orbitopathy. They can also undergo chondrogenesis and myogenesis, indicating their pluripotency[85,86]. In addition, Sven Brandau [Au: Could you please use first names on first mention?added] and colleagues demonstrated osteogenesis and neurogenesis in orbital fibroblast and orbital mesenchymal stem cells from patients with Graves' orbitopathy[86], [Au: Edits for clarity here OK? This is fine] [Au: Please reference this statement added.] although non-Graves' orbitopathy tissues were not analysed so it is unclear whether this is a truly disease-specific feature.

[Au: For the pathogenesis section, I it might be worth briefly explaining the role of adipogenesis again Added sentence] Adipogenesis, which is the differentiation process by which precursor cells develop into mature fat cells, has been demonstrated using ex vivo samples from patients with Graves' orbitopathy[87] . However, we are unaware of similar studies investigating chondrogenesis and myogenesis, which might also contribute to orbital expansion. [Au: OK this is fine?] Myogenesis might be relevant to studies reporting increased muscle volume early in disease and its correlation with Graves' orbitopathy severity, whereas orbital fat volume expanded later in disease and correlated with proptosis[88].

Analysis by flow cytometry has shown that orbital fibroblasts are largely positive for CD90 (Thy-1) and negative for CD45[85,86], [Au: Please reference this statement.done] which are positive and negative markers of mesenchymal stem cells [Au: OK? This is fine] , respectively[89]. [Au: Please reference this statement.done] Orbital fibroblasts are also particularly susceptible to inflammatory stimuli, compared with fibroblasts elsewhere. For instance, their upregulation of CD40 makes them targets for activation by CD40L on T lymphocytes[89]. [Au: Please reference this statement. done] Furthermore, TSHR-expressing T cells, which could be activated by TSHR-Ab, might further stimulate adipogenesis of orbital fibroblasts in Graves' orbitopathy through a PPARg ligand produced via upregulated cyclo-oxygenase[90] (**Figure 2**). There is potential for strategies that use the suppressive function of regulatory T cells to also prove beneficial to patients with Graves' orbitopathy [91].

Apart from orbital fibroblasts, fibrocytes, which are $CD34^{+}$ bone marrow-derived progenitor cells, migrate from the circulation into sites of inflammation and injury (**Figure 2**). They have been identified in the orbit, particularly those of patients with Graves' orbitopathy,[92,93] and reported to express two of the major thyroid autoantigens, the TSHR and thyroglobulin[94]. Studies of immune cells in the orbit have been hampered by the paucity of suitable material, but a review of available studies indicated that both CD4+ and CD8+ T cells, as well as B cells, were present in the majority of orbits examined and a 2018 [Au: Could you please define recent here by providing a timeframe?added] report suggested that the level of infiltration correlates with disease activity[95]. Macrophages are found in the orbits [Au: found in the orbits? Can you please specify done] in early disease[96], whilst monocytes and mast cells have also been identified and associated with secretion of platelet-derived growth factor (PDGF), which stimulates orbital fibroblasts proliferation and hyaluronic acid (HA) production, especially the PDGF-BB isoform, in both Graves' orbitopathy and non-Graves' orbitopathy orbital fibroblasts[97]. Mast cells also produce prostaglandins which are able to enhance adipogenesis[98]. Overall, it is increasingly recognised that there are multiple overlapping factors in the development of Graves' orbitopathy, which suggest a combination of treatments might be required.

[H2] Cellular mechanisms ***[Au: Edits to shorten heading so that it fits within our limits OK? In addition, I feel that this section works as a subsection within the pathogenesis section. Do you agree? If not, please feel free to edit my changes This is fine]***

While the role of TSHR as a Graves' orbitopathy autoantigen is widely accepted, whether the IGF-1R[69,99,100] is also a target of the autoimmune response remains controversial. Of note, however,

both TSHR and IGF-1R are expressed in orbital tissues and their expression is increased in patients with Graves' orbitopathy[72,87]. [Au: could you please reference this statement?] Since 2013 the [Au: Could you please define recent by providing a timeframe? added] establishment and replication in two laboratories of an animal model of Graves' orbitopathy, which is induced by immunisation with TSHR alone, supports the view that the TSHR is the target of orbital autoimmunity in Graves' orbitopathy[101,102].

The expression of TSHR in orbital fat was inferred by Antonio Feliciello [Au: Could you please include first names on first use? added] and colleagues [103] but confirmed using northern blot by Michele Crisp [Au: [Au: Could you please include first names on first use? added] and colleagues[104]. Immunohistochemical analysis of orbits revealed TSHR immunoreactivity, using three different monoclonal antibodies[105] . TSHR [Au: OK? This is fine] was detected in 0/20 strabismus samples, but in all 30 muscle biopsies from patients with Graves' orbitopathy, where they were associated with spindle-like cells between the extra ocular muscles; abundant mast cells were also present[106]. Several studies have illustrated that TSHR activation can impact adipogenesis; gain-of-function TSHR mutants introduced into orbital fibroblasts demonstrated that early stages of adipogenesis were enhanced[107]. Using cultured mouse embryonic stem cells, Min Lu [Au: Could you please include first name on first use? added] and colleagues showed that TSH can stimulate adipogenesis, even in the absence of adipogenic factors, suggesting that TSHR activation can initiate the early lineage commitment process[108]. In another study, Rebecca Bahn [Au: Could you please include first name on first use? added] and colleagues reported that M22, a human monoclonal TSAb, can substitute for insulin in an adipogenic cocktail[109]. However, the fact that the patient from whom M22 was derived did not have Graves' orbitopathy, and has not since developed the condition, must question how relevant it is to orbital remodelling processes.

Hyaluronan [Au: Could you please define HA here and throughout?added] is generated by 3 synthase enzymes (HAS1, HAS2 and HAS3). TSHR activation of orbital fibroblasts, by signalling through cyclic AMP (cAMP) – protein kinase A (PKA) to cAMP response element-binding protein (CREB) binding sites in the promoters of HAS1 and HAS2, increases HA production[110]. Furthermore, similar effects were obtained using a TSHR antibody devoid of TSAb activity, so-called neutral TSHR-Ab[110,111].

Lei Zhang [Au: Could you please include first name on first use?added] and colleagues also demonstrated that the processes of adipogenesis and hyaluronan production were linked in orbital adipose tissue, where hyaluronan accumulation increases during adipogenesis, but not in fat from other depots[112]. [Au: Edits to the following sentence for narrative flow OK? This is fine] The link between the pathogenesis of Graves' orbitopathy, hyaluronan production and adipogenesis was revealed in fibroblasts from orbital adipose tissue (OAT), but remarkably, was not observed in white adipose tissues (WAT), where adipogenesis leads to Hyaluronan reduction[112]. Human OAT is a neural crest derived fat depot within the orbit[113], while WAT is from mesodermal origin[114]. [Au: Please reference this statement. done] It is also interesting to note that while OAT is expanded in patients with Graves' orbitopathy, WAT shrinks due to hyperthyroidism[115]. [Au: Please reference this statement.done] Unlike WAT, OAT seems to not be a storage site for calories, and this observation is consistent with the observation that the worldwide increasing prevalence of obesity has not been associated with reports of OAT expansion[116,117].

Taken together these findings suggest a very different mechanism in OAT expansion and underlying orbital fibrosis adipogenesis. Indeed, there is a specific cell-signaling network presenting in fibroblasts from OAT distinct from that in WAT[112]. In particular mTORC1 negative-feedback in IGF1-PI3K-Protein kinase B (Akt) signaling is absent in OAT-orbital fibroblasts, but present in WAT [112]. Studies have also shown TSHR–PKA, IGF1R–PI3K–Akt and mTORC1 signalling worked together in the pathogenesis of Graves' orbitopathy [99,109,110,118-120].

Whilst data are strong for the effect of TSHR in Graves' orbitopathy, IGF-1R is also likely to have a key role in the disease. [Au: I have made some edits to this sentence to improve the narrative flow. Please check that I have not altered your intended meaning and feel free to alter my changes if you think I have. This is fine] This hypothesis [Au: OK? This is fine] is exemplified by the 2017 trial which [Au: Can you please define recently here by providing a timeframe?added] reported dramatic reduction in proptosis in patients with Graves' orbitopathy who had been treated with Teprotumumab[73], which suggests a central role for signalling via IGF-1R in Graves' orbitopathy pathogenesis. This finding has been further illustrated [Au: Edit OK? This is fine] by studies that have highlighted the importance of downstream factors of IGF1–PI3K signalling and revealed that nuclear Forkhead transcriptional factors, FOXOs, serve as convergence points for TSHR and IGF-1R signalling pathways in Graves' orbitopathy[121,122]. Specifically, FOXO1 and FOXO3a served as repressors, which protect orbital fibroblasts from excessive adipogenesis and marked over-production of HA, respectively[121]. In addition, an old drug, trifluoperazine

hydrochloride, which enhances FOXO repressors abolished adipogenesis and HA production in OAT-orbital fibroblasts[121]. Unfortunately this drug has substantial adverse effects including liver damage, bradycardia and dyskinesia [Au: Could you please provide one or two examples? done] and therefore has no role in thyroid eye disease[123]. Alternative drugs targeting FOXO transcription factors [Au: What is "this" referring to here specifically?changed] might therefore replicate the recent clinical trial with teprotumumab showing reduced proptosis of severe Graves' orbitopathy patients by inhibiting IGF-1R signalling[73] (**Figure 2**). Taken together, FOXOs could be an alternative non-immunosuppressive therapeutic target that would potentially reset tissue remodelling and pathological orbital expansion in Graves' orbitopathy with less cost than teprotumumab. Furthermore, they may have wider effects on Graves' orbitopathy pathology.

[H1] Two autoantigens, possible cross-talk?

Patients with severe Graves' orbitopathy have an increased risk of relapsing hyperthyroidism and are unlikely to remain in remission[124]. Although several authors have reported correlations between TSAb titre and Graves' orbitopathy prevalence and/or severity[125-127], the fact that not all patients with Graves' orbitopathy have TSHR-Abs with stimulating activity on the TSHR (TSHRAbs [Au: OK? This is fine] activate PKA–cAMP cascade) has led to two differing conclusions. The first is that TSHR-Abs signal might exist that signal to other cascades that might exist and the second that there is an additional autoantigen[111,128]. [Au: Is this what you mean? Please edit my changes if I have misunderstood you modified edit slightly]

IGF1R has been the focus of considerable attention following the early demonstration that IgGs in patients with Graves' orbitopathy are able to inhibit binding of IGF1 and therefore is analogous to TSH-binding inhibitor immunoglobulin (TBII) in the thyroid[100]. Terry Smith [Au: Could you please use first name on first use? added] and colleagues have reported Graves' orbitopathy IgGs with a wide range of IGF1R 'stimulating' activities (via IGF-1R auto phosphorylation), from enhanced orbital fibroblasts proliferation to increased secretion of inflammatory cytokines and elevated production of GAGs[69,70]. However, reports from other authors have failed to demonstrate that; autoantibodies are able to auto-phosphorylate the IGF-1R; that IGF-1R autoantibodies simply binding the receptor are more abundant in patients Graves' orbitopathy than in healthy controls[129,130]; or that autoantibodies are more prevalent in patients with Graves' disease [Au: OK?this is fine] with Graves' orbitopathy than free of eye disease[130].

An alternative to direct activation of the IGF-1R, inducing Graves' orbitopathy is the possibility of a synergistic effect of IGF-1R and TSHR. Christine Krieger [Au: Could you please include first name on first use?added] and colleagues[120] described synergistic actions of TSH and IGF-1 in stimulating HA, although the cells were in a semi-adipogenic medium likely to increase TSHR expression. The group also explored the biphasic dose-response of M22 (a human [Au: OK? This is fine] monoclonal TSHR-Ab with stimulating activity that does not induce IGF-1R autophosphorylation) and found that even though a TSHR antagonist inhibited both phases, an IGF-1R antagonist inhibited only the higher potency phase, leading to their notion of cross-talk. In subsequent studies the group proposed combination therapy using low dose TSHR and IGF-1R antagonists as IGF-1R antagonism alone (as in Smith and colleagues teprotumumab trial)[73] might not benefit patients with Graves' orbitopathy who have high TSAb levels. One could therefore envisage alternative signalling cascades to explain the TSHR–IGF-1R cross-talk (**Figure 2**), but another explanation is that activation of IGF1R by IGF1 (increased in Graves' disease and Graves' orbitopathy) is able to upregulate TSHR expression – as has been reported in the thyroid [70].

[H2] IL17 and a possible role for the microbiome *[Au: I believe that this section fits within the pathogenesis section still. Please feel to make this a Level 1 (H1) heading if you disagree this is fine]*

One of the major problems facing patients with [Au: Edit OK? This is fine] Graves' orbitopathy is fibrosis, which results in permanent remodelling of the orbit. Fibrosis follows myofibroblast differentiation of CD90–Thy-1 positive orbital fibroblasts, which are stimulated by transforming growth factor (TGF)-beta. In 2016 [Au: Could you please define "recently" here by providing a timeframe? added] a role for Th17 cells has been implicated in fibrosis, in line with other autoimmune conditions in which Th17 cells have been found in autoimmune lesions, such as multiple sclerosis' plaques. Bin Li [Au: Could you please use first name on first mention? Bin Li is full name] and colleagues work[131] showed a significantly ($p<0.01$) higher proportion of IL-17A-producing T cells in patients with Graves' orbitopathy compared with healthy controls [Au: Compared with who? In addition, can you please include a P value for the use of "significantly" here? added] and the recruitment of both CD4 and CD8 T cells in Graves' orbitopathy orbits. In addition, orbital tissues from patients with Graves' orbitopathy expressed more IL-17A receptor, IL-17A, and its related cytokines (**Figure 2**), and the authors noted severe fibrotic change compared with normal controls[132]. A product isolated from traditional Chinese medicine (vialinin A) was able to inhibit RORγt, a transcription factor which drives Th17 differentiation

and thus reduce [Au: The numbers of what, specifically?changed] numbers of TH17 cells[131]. This finding might lead to additional therapies to limit the disfigurement observed in Graves' orbitopathy.

[H1] Animal models of Graves' orbitopathy

Whilst considerable progress has been made in understanding the pathogenetic mechanisms operating at the end stages of disease, relatively little is known about the factors that cause loss of immune tolerance and trigger disease onset. Animal models can be invaluable in this context and numerous attempts have been made to develop a robust model of induced Graves' orbitopathy in mice. [Au: Edit to the following sentence OK? Minor changes] Many of these models, however, were only able to induce Graves' disease, with no signs of Graves' orbitopathy[133-135]. In one instance researchers developed a BALB/c based mouse model that had some aspects of Graves' orbitopathy [136], but this was not reproducible in different centres, which suggests a role for environmental variables, including micro-organisms, interfering with Graves' orbitopathy pathogenesis[137].

Since 2011 [Au: Could you please define "recently" by providing a timeframe?added] , success has been achieved using an expression plasmid for the TSHR-A subunit that was introduced using electroporation in female BALB/c mice[101,102,138]. A notable proportion of the mice developed a Graves' orbitopathy-like disease, with evidence of fibrosis[138], inflammation[101] and enhanced adipogenesis[101,102]. Considering the previous challenges in reproducing Graves' orbitopathy animal models in different environments [137] this model has been reproduced in parallel in two independent laboratories (UK and Germany), following the same immunisation procedure[102]. This finding provides strong evidence for the TSH-receptor being a target autoantigen in the Graves' orbitopathy. Interestingly, however, while the induced Graves' orbitopathy mice had similar features in both centres (enhanced adipogenesis and atrophy of ocular muscles), there were some differences, with a marked proportion of mice in centre 1 (UK) developing hyperthyroidism, while all mice in centre 2 (Germany) remained euthyroid[102]. [Au: Please reference this statement. added] Some of the discrepancies could be attributed to differences in gut microbiota composition across the two centres, as shown in a subsequent study evaluating disease-associated microbial taxonomies[139]. [Au: Please reference this statement.added]

For example, marked differences in α-diversity, β-diversity and in the taxonomic profiles were observed between TSHR-immunized mice in the two centres (for example the genus Lactobacillus was more abundant in Germany, while Bacteroides and Bifidobacterium counts were more abundant in UK). The gut microbiota was also compared in TSHR and βgal untreated control mice in Germany where the authors noted a shift in the TSHR immunized mice bacterial communities (β-diversity weighted Unifrac) together with a significant ($p<0.005$) [Au: could you please provide a P value for the use of significant here?added] positive correlation between the Firmicutes phylum counts and orbital-adipogenesis[139]. This interplay between gut microbiota and disease progression might reveal additional novel insights into the pathophysiology of Graves' orbitopathy.

[H1] Conclusion

Developments in our understanding of the pathogenesis and natural history of Graves' orbitopathy over the past five years [Au: addition of 5 years to provide timeframe OK? This is fine] have led to renewed focus on how to optimise management of Graves' orbitopathy. It is increasingly clear that early interventions are likely to substantially alter the course of the disease and improve long term outcomes and associated morbidity. Furthermore, with the identification of multiple therapeutic targets (**Figure 2; table 3**), the synergistic potential of combination therapy has emerged. However, confirmatory trials are still warranted and require a comparison with current Graves' orbitopathy therapy for moderate to severe disease. If confirmed this finding will open the door to a new era of agents that are either non-immunosuppressive or have very selective effects on the immune system. Of particular importance for current patients, however, is that we are yet to identify a non-surgical intervention that can improve outcomes in the 'burnt-out' phase[140]. Future trials should also evaluate outcomes beyond one year, to confirm that early intervention reduces long term orbital deformity and the need for rehabilitative surgery and improve long term quality-of-life.

Author contributions [Au: OK? Please feel free to edit if not correct]

All authors equally contributed to all aspects of the article. Drafting, critically evaluating the content and correcting the manuscript was done by all co-authors. PT and LZ share first and GJK and ML senior authorship

Competing interests [Au: OK? This is fine]

The Johannes Gutenberg University (JGU) Medical Center and the JGU Thyroid Lab received research grants from Novartis, Germany and River Vision, USA when performing the MINGO and Teprotumumab trials.

Publisher's note

Box 1 Key validated Graves' orbitopathy activity and severity scores

Scores Assessing Disease Activity

- Clinical Activity Score – based on pain, redness, and swelling swelling.The baseline CAS assessment is scored out of 7. Repeat clinical activity score assessments are scored out of 10 points as this includes changes in proptosis, eye movement and visual acuity[19]. [Au: Please reference this statement.done]

- VISA-I – Inflammation component based on caruncular oedema, chemosis, conjunctival redness, lid redness, lid oedema and retrobulbar ache[18] [Au: Please reference this statement added. Could you please also explain what is meant by caruncular (G), chemosis (G) and retrobulbar(G) for our non-specialist readers? Added to glossary]

Objective Scores Assessing Graves' orbitopathy Severity

- EUGOGO – based on whether sight-threatening Graves' orbitopathy (requiring immediate intervention (dysthyroid optic neuropathy and/or corneal breakdown), moderate-to-severe Graves' orbitopathy (requiring immunosuppression if active or surgical intervention if inactive patients usually have one or more of lid retraction ≥ 2mm, moderate or severe soft tissue involvement, exophthalmos > 3mm constant or inconstant diplopia) or mild Graves' orbitopathy where it is difficult to justify immunosuppressive or surgical therapy (minor lid retraction <2mm, mild soft tissue involvement, exophthalmos <3mm, transient or no diplopia, and corneal exposure responsive to lubricants) [23] [Au: Please reference this statement. Done]

- VISA–VSA vision, strabismus, and appearance components. Vision is assessed by acuity, colour vision and fields. Strabismus is assessed by diplopia and motility restriction and appearance is assessed by appearance concerns and evidence of ocular exposure[8]. [Au: Please reference this statement.done]

- Ophthalmopathy Index – a 25 point score covering soft tissue inflammation, exophthalmos, palpebral aperture, diplopia, corneal involvement and evidence of optic neuropathy[22]. [Au: Please reference this statement. done]

- NOSPECS – a mnemonic acronym for 'no symptoms and/or signs, only signs, soft tissue involvement, proptosis, extraocular muscle involvement, corneal involvement, sight loss[20,21] [Au: Please reference this statement.done]

- Graves Ophthalmopathy Quality of Life (GOQOL), introduced by EUGOGO, is a disease specific quality of life score focussing on visual function and cosmetic visual appearance[26-28].

[Au: Does this mean there aren't any official scores? If so, I would suggest removing this heading agreed added GOQOL and removed modified the section

Box 2: TEAMeD *5* Step approach[82] [Au: Please reference this statement. done]

- Diagnose Graves' disease accurately (measure TSHR-Ab)
- Screen all patients with Graves' disease for Graves' orbitopathy at each visit
- Alert all patients with Grave's disease to the risk of Graves' orbitopathy
- Prevent Graves' orbitopathy - encourage smoking cessation, achieve and maintain euthyroidism quickly, avoid radioactive iodine (RAI) in active Graves' orbitopathy, avoid hypothyroidism after RAI
- Refer moderate and/or severe Graves' orbitopathy to a specialist multidisciplinary clinic early

Box 3: Treatments used for Graves' orbitopathy

Medical – immunosuppressive and immunomodulatory agents[1,2,51,81,82] *[Au: Please reference this statement.done]*

- Glucocorticoids
- Orbital radiotherapy
- Mycophenolate
- Cyclosporine
- Teprotumumab
- Rituximab
- Tocilizumab,
- Emerging biological agents, such as Belimumab

Medical non immunosuppressive[43] *[Au: Please reference this statement.done]*

- Selenium

Surgery[2,141] *[Au: Please reference this statement.done]*

- Orbital bony decompression
- Squint surgery
- Lid surgery including Blepharoplasty or other reconstructive surgery

BOX 4: Rehabilitative Surgical management of Graves' orbitopathy

A variety of surgical options are available to treat Graves' orbitopathy, depending on the nature of changes to the orbit. Lateral wall decompression, is the procedure of choice for the correction of proptosis of the globe if projection is <5mm. Persistent diplopia often requires squint surgery. The goals of surgery are to maintain binocular single vision in the primary position and on downgaze, meaning that patients will often have residual diplopia in other directions of gaze[142]. Finally, eyelid surgery in the form of blepharoplasty, lid lowering, midface lifting and brow fat pad reduction can be performed to remove bags and tighten the skin. Upper lid retraction can also be addressed with levator recession surgery[143]. [Au: Please reference this statement. done]

Table 1 Summary of key clinical trials since 2015 [Au: To define "recent" could you please provide a timeframe? In addition, I have flipped the table so that it fits within our style. I have left the text the same, unless marked with an Au:, but did remove the name of the authors to save space. Changes are fine have altered title of table]

Agents studied	Design	N (year)	Countries	Follow up	Key outcome assessed	Key findings of trial	Refs
Tocilizumab	Multi-centre double masked randomised trial	32 (2018)	Spain	40 weeks	Clinical activity score, EUGOGO ophthalmic score, Exophthalmos	In glucocorticoid resistant GO tocilizumab resulted in substantial reduction in Clinical activity score, EUGOGO ophthalmic score and exophthalmos	[81]
Teprotumumab	Multicentre Randomised placebo controlled trial	88 (2017)	USA, UK, Germany and Italy [Au: OK? fine]	52 weeks	Proptosis, Clinical activity score	Substantial improvement in both proptosis and Clinical activity score	[73]
Azathioprine, orbital radiotherapy	Factorial design masked randomised control trial.	126 (2018)	UK	24 weeks	Composite outcome	No benefit observed with radiotherapy. Potential benefit observed with Azathioprine	[29]
Mycophenolate sodium	Multicentre Observer masked randomised controlled trial IV Glucocorticoids vs IV Glucocorticoids plus Mycophenolate sodium	164 (2018)	Germany and Italy	12 weeks	Composite outcome	Addition of mycophenolate sodium significantly improved response rate at weeks 24 and 36	[30]
Mycophenolate Mofetil (MMF)	Randomised trial of MMF vs IV methyl prednisolone and oral Glucocorticoids	174 (2016)	China	Treatment of 24 Weeks	Clinical activity score, proptosis, and diplopia	MMF superior to IV and oral glucocorticoids with regard to clinical activity score proptosis, diplopia and safety	[48]
Rituximab	Randomised double masked - IV methyl prednisolone vs Rituximab	31 (2015)	Italy	52 weeks	Clinical activity score	Rituximab may be superior to IV methyl prednisolone	[76]†
Rituximab	Randomised double masked, placebo-controlled trial Rituximab versus placebo	24 (2015)	USA	52 weeks	Clinical activity score	No apparent benefit of Rituximab vs placebo	[75]†

N= Number of participants, IV = intravenous, MMF = Mycophenolate mofetil. †Differences in these trials may relate to Rituximab being only effective in early active disease

Table 2 Summary of newer [Au: newer requires a comparator, could this be changed to the date at which these agents were developed?] agent dosing and key monitoring requirements

Agent	Typical Doses	Key monitoring and/or exclusion
Mycophenolate[30,48,65]	360mg twice a day orally for 24 weeks (mycophenolate sodium) 1 g twice a day for 24 weeks (mycophenolate mofetil)	Monitor FBC, LFT
Teprotumumab[73]	1 intravenous infusion of 10mg/Kg then 7 infusions of 20mg/Kg. Infusion every 3 weeks. Total treatment 24 weeks	Monitor glucose, LFT
Tocilizumab [79,81]	Intravenous infusions of 8mg/Kg every 4 weeks for 12 weeks.	Monitor lipid profile, LFT, FBC and for demyelinating disorders.

FBC = Full blood count
LFT = Liver function test

Table 3 Current and Potential therapeutic targets

Target	Class of Therapy	Mechanism of action	Evidence of benefit	Study
Fibroblasts	Glucocorticoids	Reduced prostaglandin secretion, fibroblast activity, glycosaminoglycan production Pro-inflammatory cytokines	+++	Zang[13] [Au: Can you please reference them here?added]
Fibroblasts	Prostaglandin F2 alpha (Bimatoprost)		Unclear	Draman[83]
Fibroblasts	Mycophenolate sodium/Mycophenolate mofetil/Azathioprine	Inhibition of PI3Kinase and mTOR pathways	+	Rajendram, Taylor [29] Kahaly [30] Ye[48]
Fibroblasts	IGF-1 Receptor monoclonal antibody (Teprotumumab)	Reduced PI3K activation and TSHR cross talk	+++	Smith[73]
T and B cells	Glucocorticoids	Inhibits proliferation and cytokine production	+++	Zang[13,53] [Au: Can you please reference them here?added]
T and B cells	Orbital radiotherapy	Depletion, inhibits proliferation	+ (as monotherapy)	Prummel[56]
T and B cells	Mycophenolate sodium/Mycophenolate mofetil/Azathioprine	Inhibits proliferation	+	Rajendram, Taylor [29] Kahaly [30] Ye[48]
B cells	CD20 monoclonal antibody (Rituximab)	B cell depletion, reduced antigen presentation	++	Stan[75] Salvi[76]
IL6	Anti-IL6 receptor monoclonal antibody (Tocilizumab)	Inhibits the pro-inflammatory cytokine IL-6	++	Perez-Moreiras[79,81]
Selenoproteins	Anti-oxidant	Selenium	++†	Marcocci [43]

† evidence-based use of selenium is limited to patients with newly onset, mild and active GO

Figure Titles and legends

Figure 1 Proposed management of Graves' Orbitopathy

These guidelines have been adapted from the 2016 European Thyroid Association (ETA) Guidelines for the management of Graves' Orbitopathy[23]. These recommendations were also integrated in the 2018 ETA guidelines for the management of Graves' hyperthyroidism[50].

Figure 2 Overview of potential therapeutic targets in Graves' Orbitopathy

Disease mechanisms of Graves' orbitopathy are indicated by dashed lines and pathogenesis red boxes. Existing and novel therapeutic candidates are represented by blue boxes and solid lines (see Table 2 for further information on mechanisms of action). *Potential therapeutic targets, Rapamycin, inhibitor of mTOR (mammalian target of rapamycin)[118]. TFP (trifluoperazine hydrochloride) to recruit FOXO repressors[121]. Other abbreviations: orbital adipose tissues (OAT); TSHR (T); IGF1 (I); TSHR auto-antibodies (TRAB); protein kinase A (PKA); phosphoinositide 3-kinase (PI3K); Tumor necrosis factor (TNF); Interleukin 6 (IL-6); small molecule (SM) antagonist; TSHR blocking antibodies (TBAB); Teprotumumab (TMB); Mycophenolate mofetil (MMF); Azathioprine (AZA); hyaluronan production (HA).

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Glossary:

Proptosis [G] Abnormal protrusion of the eyeball.

Diplopia [G] Double Vision.

Masked [G] Same as blinded in clinical trials, observer assessing outcomes does not know treatment allocation, masked is used instead of blinded in ophthalmology trials to not alarm patients !

Caruncular oedema [G] Oedema of the small, pink, globular nodule at the inner corner (the medial canthus) of the eye.

Chemosis [G] Swelling and oedema of the conjunctiva

Retrobulbar [G] Behind the eyeball